PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 62310WO (71699)	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US05/09391	International filing date (*day/month/year*) 22 March 2005 (22.03.2005)	(Earliest) Priority Date (*day/month/year*) 22 March 2004 (22.03.2004)
Applicant THE JOHNS HOPKINS UNIVERSITY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (See Box No. II)
3. ☒ **Unity of invention is lacking** (See Box No. III)
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/09391

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 28-74 as they apply to detection of viral vairants

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/09391

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) : C12Q 1/68; C12P 19/34

US CL : 435/6, 91.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6. 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	SCHOUTEN, J.P. et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Research. 2002, Vol. 30, No. 12, e57, pp.1-13.	1, 30-39, 41, 43-45, 49-50, 52, 56-58, 60, 63-66, 68, 69, 71-74 --- 5, 40, 42
X --- Y	WO 97/45559 (BELGRADER et al) CORNELL RESEARCH FOUNDATION, INC. 1997-05-27. entire document.	1-4, 30-39, 43-46, 49-52, 54-57, 59-61, 63-66, 68, 71-74 --- 28, 29, 47, 48, 53, 62, 70
Y	BECK, I. et al. Genotyping kits for the detection of HIV-1 pol drug resistance mutations by an oligonucleotide ligation assay. NIH AIDS Research and Reference Reagent Program. April 4, 2003. pages 1-24.	5

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 January 2006 (19.01.2006)

Date of mailing of the international search report

06 APR 2006

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (571) 273-3201

Authorized officer

Stephen Kapushoc

Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/09391

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAPALA, J. et al. Pooling cervical swabs and testing by ligase chain reaction are accurate and cost-saving strategies for diagnosis of Chlamydia trachomatis. Journal of Clinical Microbiology. July 2000, pp.248-2483.	28, 29, 47
Y	US 5,185,243 (ULLMAN E.F. et al) 9 Feb. 1993 (09.02.1993). entire document, especially column 9, lines 59-68.	40, 42
Y	WHITCOMBE, D. et al. Detection of PCR products using self-probing amplicons and fluorescence. Nature Biotechnology. August 1999, Vol. 17, pages 804-807.	48
Y	FAVIS, R. et al. Universal DNA array detection of small insertions and deletions in BRCA1 and BRCA2. Nature Biotechnology. May 2000, Vol. 18, pages 561-564.	53, 62
Y	US 5,210,015 (GELFAND, D.H. et al) 11 May 1993 (11.05.1993) entire document.	67
Y	ABRAVAYA K. et al. Detection of point mutations with a modiied ligase chain reaction (Gap-LCR). Nucleic Acids Research. 1995, Vol. 23, No. 4, pages 675-682.	70

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claims 1-74, drawn to methods for detecting infectious disease variants, differentiating pancreatic cancer from chronic pancreatitis, diagnosing a disease, forensic identification, identifying a genetic change, or detecting a nucleic acid difference.

Group II, claims 75 ad 76, drawn to kits comprising primers suitable for ligation to one another.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species regarding the application of the claimed methods of group I are as follows:

1. detecting an infectious disease minor variant that is HIV
2. detecting an infectious disease minor variant that is HBV
3. detecting an infectious disease minor variant that is HCV
4. detecting an infectious disease minor variant that is CMV
5. detecting an infectious disease minor variant that is influenza
6. detecting an infectious disease minor variant that is HSV
7. detecting an infectious disease minor variant that is RSV
8. detecting an infectious disease minor variant that is VZV
9. differentiating pancreatic cancer from chronic pancreatitis
10.-38. diagnosing any one of the diseases as listed in claim 16
39.-70. diagnosing any one of the cancers as listed in claim 17
71. forensic identification
72. identifying an uncommon genetic change

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

As detailed above, the common technical feature linking the species is the method that is not an advancement over the prior art.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The method of claim 1 is not an advancement over the prior art of Schouten et al (Nucleic acids research (2002) Vol. 30, No. 12, e57, pp.1-13). Schouten et al teaches a method for multiplex ligation-dependent probe amplification (Figure 2, p. 4) which includes: i) contacting a target nucleic acid with a pair of oligonucleotides, where each oligonucleotide has a gene specific region and a primer region, and one oligonucleotide contains a detector region (referred to in the reference as a stuffer sequence), ii) subjecting the mixture to a ligation reaction, and iii) amplifying the ligation products by PCR.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/09391

Continuation of B. FIELDS SEARCHED Item 3:
PubMed, STN search of caplus, agricola, caba, biosis. EAST search of patent literature. Search terms: Ligation dependent mutation detection, ligase chain reaction, ligation probes